Neuroactive steroids and anxiety disorders

Jean-Michel Le Mellédo, MD; Glen B. Baker, PhD, DSc

Department of Psychiatry, University of Alberta, Edmonton, Alta.

Introduction

The classic mechanism of action of steroid hormones involves binding to their respective intracellular receptors, which act as transcription factors in the regulation of gene expression. The ensuing effects, described as the genomic effects of steroids, are relatively slow. Evidence that some steroids, through interaction with certain neurotransmitter receptors, can alter neuronal excitability at the cell surface has led to the description of the rapid membrane effects of steroids. The term "neuroactive steroid" (NAS) has been coined for steroids with these particular properties.[1] Diverse mechanisms for rapid steroid effects have now been described, and the nongenomic effects of NASs may involve γ-aminobutyric acid (GABA) receptors, glutamate receptors, nicotinic acetylcholine receptors, sigma receptors, 5-HT_3 receptors, or voltage- or non-voltage-gated calcium channels. These various mechanisms are reportedly involved in functions as diverse as sleep, anxiety, seizure activity, aggressive behaviour, response to stress, and neuronal regeneration and protection, as well as in learning and memory.[2]

Neuroactive steroids, the $GABA_A$ receptor complex and anxiety

The bulk of research on the role of NASs in anxiety and stress involves the $GABA_A$ receptor complex, at which several NASs act as positive or negative allosteric modulators. NASs that affect $GABA_A$ receptor function include progesterone derivatives such as allopregnanolone (ALLO; 3α,5α-tetrahydroprogesterone), epiallopregnanolone (3β,5α-tetrahydroprogesterone), pregnanolone (3α,5β-tetrahydroprogesterone), and tetrahydrodeoxycorticosterone (3α,5α-THDOC), as well as pregnenolone (PREG), pregnenolone sulfate (PREGS), dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Such modulation suggests a direct binding site (or sites) for the NAS on the $GABA_A$ receptor, although such sites have not yet been unequivocally demonstrated. These NASs can be synthesized de novo in the nervous system (in which case they are called neurosteroids[3]), the adrenals, the gonads and the placenta. Of these NASs, only 3α,5α-THDOC is not considered a neurosteroid stricto sensu because, although it is produced in the brain from deoxycorticosterone, it becomes undetectable in that organ after adrenalectomy. Enzymes involved in the biosynthesis of these NASs, such as 5α-reductase and 3α-hydroxysteroid oxidoreductase, are found in key neuroanatomic structures involved in anxiety such as the amygdala and the hippocampus.[4]

The endogenous 3α-hydroxy ring A-reduced steroids such as ALLO and 3α,5α-THDOC are among the most potent ligands of the $GABA_A$ receptor, with affinities equal to or greater than those of other known ligands such as benzodiazepines or barbiturates.[5] In

Correspondence to: Dr. Jean-Michel Le Mellédo, Department of Psychiatry, University of Alberta, Edmonton AB T6G 2R7; fax 780 407-6672; jean-michel.lemelledo@ualberta.ca

Medical subject headings: anxiety disorders; steroids; progesterone; pregnanolone; pregnenolone; prasterone; receptors, GABA.

J Psychiatry Neurosci 2002;27(3):161-5.

Submitted: Sept. 4, 2001
Accepted: Mar. 5, 2002

animal models of anxiety, positive modulation of the $GABA_A$ receptor has been associated with anxiolytic activity, whereas negative modulation has been associated with anxiogenic activity.[6] Indeed, in most, but not all, studies involving several animal models, peripheral and central administration of ALLO, 3α,5α-THDOC and, to a lesser extent, PREG has resulted in significant anxiolytic activity.[6,7] ALLO also appears to be responsible for the observed anxiolytic activity of progesterone. Bitran et al[7] showed that administration of progesterone is associated with increases in serum and cortical levels of ALLO and that the anxiolytic activity of progesterone was due to its conversion to ALLO.

The effects on anxiety of PREG, PREGS, DHEA and DHEAS are less clear than those of ALLO. PREG has been reported to induce anxiogenic activity in mice tested in the plus-maze model.[8] In the same study, PREGS produced a biphasic action, with a low dose (in the nanomolar range) inducing anxiolytic effects and a high dose (in the micromolar range) an anxiogenic response. Contradictory results have been obtained with PREGS, which may reflect the mixed agonist–antagonist profile observed at the level of $GABA_A$ receptor function.[9] However, these results could also be explained by the positive allosteric modulator activity of PREGS at the level of the *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptor, given that antagonist activity at the level of this receptor is associated with anxiolytic activity.[10] Observed anxiogenic activity of DHEAS in mice undergoing the mirror chamber behavioural test is consistent with its antagonist properties at the level of the $GABA_A$ receptor.[11] However, in studies with the plus-maze model in mice, DHEA and DHEAS both produced anxiolytic effects.[12] Interestingly, DHEAS can block the anxiolytic activity of dizocilpine, an NMDA receptor antagonist, in the mirror chamber test, which suggests that the effects of DHEA and DHEAS on anxiety might be mediated through mechanisms independent of $GABA_A$ receptor activity.[11]

Clinical studies on neuroactive steroids

NASs, particularly ALLO and pregnanolone, are reportedly decreased in both the cerebrospinal fluid (CSF) and the plasma of untreated patients with major depressive episode.[13,14] Antidepressant treatment with the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and fluvoxamine, also used to treat anxiety disorders, normalized the ALLO and pregnanolone content of the CSF, and there was a correlation between improvements in symptoms and increase in CSF levels of ALLO and pregnanolone.[15] Interestingly, Romeo et al[13] found a chronological association between SSRI-induced increases in plasma ALLO and the classically delayed clinical response to antidepressant treatment (also true for the clinical response to the anxiolytic activity of SSRIs). The SSRIs have also been reported to alter the enzymatic activity of the 3α-hydroxysteroid oxidoreductase.[15,16]

To the best of our knowledge, we have performed the first studies assessing plasma levels of NAS derivatives of progesterone in patients with anxiety disorders, namely generalized anxiety disorder, generalized social phobia and panic disorder. Contrary to our hypotheses, we did not find any clear differences in baseline plasma levels of ALLO between patients with generalized anxiety disorder,[17] generalized social phobia[18] or panic disorder (unpublished data) and normal subjects. This last result contradicts a recently published paper which described higher ALLO levels in patients with panic disorder.[19] Interestingly, we found that patients with generalized anxiety disorder or generalized social phobia but not panic disorder had lower plasma levels of PREGS than healthy volunteers. However, we did not find any correlation between plasma levels of PREGS and the severity of symptoms, as assessed with the Hamilton Anxiety and the Liebowitz Social Anxiety scales in patients with generalized anxiety disorder or generalized social phobia respectively. Given the lack of correlation between PREGS levels and symptom severity, the lower levels of PREGS may represent insufficiency of a compensatory mechanism. Since PREGS is anxiolytic at low doses (through agonist activity at the level of the $GABA_A$ receptor) but anxiogenic at high doses (through antagonist activity at the level of the same receptor), the observed lower levels of PREGS could be interpreted as an attempt to cope with pathologic anxiety by counteracting the detrimental antagonist activity of PREGS at the level of the $GABA_A$ receptor. In addition, data from animal studies suggest that the anxiogenic activity of PREGS can also be mediated by its positive allosteric modulator activity at the level of the NMDA excitatory amino acid receptor,[20] which is in accordance with the anxiogenic activity associated with increased glutamatergic activity.[10]

If the lack of pathologic anxiety is considered the result of a homeostatic balance between endogenous

anxiolytic and anxiogenic agents and more particularly is determined to represent an equilibrium between GABAergic and glutamatergic activity,[8] the body would be able to maintain homeostasis through fluctuations in the intensity of GABAergic and glutamatergic function. In the context of pathologic anxiety, lower production of PREGS could be interpreted as a homeostatic attempt to decrease anxiogenic activity through a lesser negative modulation of the $GABA_A$ receptor and a lesser positive modulation of the NMDA receptor. Spivak et al[21] found that plasma levels of DHEA and DHEAS were higher than normal in male patients with combat-related posttraumatic stress disorder; however, we did not find any such changes in patients with generalized anxiety disorder, generalized social phobia or panic disorder.

Neuroactive steroids and the hypothalamic-pituitary-adrenal axis

Several animal studies have shown that various stressors induce a delayed increase in brain and plasma concentrations of ALLO and 3α,5α -THDOC, 2 positive modulators of the $GABA_A$ receptor.[22,23] These stress paradigms were associated with an initial increase in binding of *tert*-butylbicyclophosphorothionate labelled with sulphur-35, a sensitive marker of $GABA_A$ receptor function; the increased binding was interpreted by Barbaccia et al[23] as a consequence of decreased GABAergic transmission. One of the stress paradigms used in male rats was inhalation of carbon dioxide, a challenge known to induce anxiety-like behaviour in rats and panic attacks in humans.[23] Our pilot data in men with panic disorder and healthy volunteers, which showed a strong trend toward greater levels of ALLO (almost reaching statistical significance [$p = 0.08$]), suggest that challenge with the panicogenic agent pentagastrin may also induce a delayed release of ALLO in plasma.[24]

This stress- or panic-induced release of NASs has been speculated to represent an endogenous homeostatic mechanism for restoring the GABAergic system after stress[23] as well as for restoring normal activity of the hypothalamic-pituitary-adrenal (HPA) axis. Interpretation of this delayed increase in ALLO (and likely 3α,5α-THDOC) as a protective homeostatic mechanism against stress is supported by the effects of ALLO and 3α,5α-THDOC on HPA axis activity and the well-known inhibitory effects of $GABA_A$ receptor activity on HPA axis activity.[25] For example, peripheral administration of ALLO and 3α,5α-THDOC to rats dampened the activity of the HPA axis in response to stress.[26,27]

Subcutaneous administration of ALLO lessened the release of corticosterone in response to air puffs (a stress test) in adult male rats and decreased gene transcription of arginine vasopressin, a secretagogue of adrenocorticotropic hormone, in the hypothalamus.[27] Subcutaneous administration of 3α,5α-THDOC attenuated the long-lasting HPA axis-related alterations -associated with maternal separation in infant male rats. Indeed, administration of 3α,5α-THDOC to male rat pups before separation from their mothers counteracted the exaggerated adrenocortical (cortiscosterone) response to emotional stress, the decreased responsiveness to the suppressive action of dexamethasone, the increased levels of corticotropin-releasing hormone messenger RNA in the paraventricular nucleus and the diminished numbers of glucocorticoid receptor-encoding transcripts in the hippocampus.[28] Such inhibitory effects of these NAS $GABA_A$ agonists are not surprising, since other positive allosteric modulators of the $GABA_A$ receptor such as benzodiazepines inhibit baseline and post-challenge HPA axis activity in rodents as well as in healthy human volunteers and patients with panic disorder.[29-31]

We are therefore proposing that there is a full-loop homeostatic control mechanism between the HPA axis and the NAS system during stress, with a paramount negative feedback of NASs released after stress on the increased HPA axis activity displayed during stress. This interpretation fits the relatively new concept of allostasis and allostatic load described by McEwen and Seeman[32] and suggests that NASs may play an important role in this multidimensional extension of the stress concept. This concept is based on the fact that release of certain hormonal mediators such as glucocorticoids and catecholamines are essential for adaptation, maintenance of homeostatis and survival in the short term in response to acute stress (allostasis), but if this release of "stress" hormones persists, they might be harmful, inducing a disease process (allostatic load). We believe that it is essential to continue investigating the release of NASs in response to stress in humans because data suggest that such release, particularly the release of positive allosteric modulators of the $GABA_A$ receptor, contribute (in association with, for example, cortisol negative feedback) to the extinction of the allostatic mechanisms after termination of an acute stressor. This release of NASs therefore prevents the devel-

opment of deleterious effects (allostatic load) that would be associated with the persistence of the biological response to stress and, hence, prevents its deleterious effect on health.

Conclusions

There are now preliminary clinical data to support the results of animal investigations that have suggested a role of NASs in anxiety and therefore a potential role for NAS analogues in the treatment of anxiety disorders. Our results and those of others suggest that anxiety disorders have in common some NAS dysregulations but that each anxiety disorder may display its own specific pattern of dysregulation. A global interpretation of the role of human NASs in anxiety disorders will be facilitated by extensive measurements of various NASs since the effect of a decrease in one NAS may be offset by an increase in another one with similar activity at the level of the $GABA_A$ receptor.[33] Given the efficacy of SSRIs in the treatment of most anxiety disorders and their ability to alter the enzyme kinetics involved in NAS metabolism, it will also be important to assess whether successful treatment with SSRIs is associated with normalization of the NAS dysregulation observed in patients with anxiety disorders.

The crucial role that NASs seem to play in preventing a chronic allostatic load suggests that their importance extends beyond mental health and that NAS dysregulation may be a unique link in the now well described association between physical health and mental health.

Acknowledgements: We are grateful to the Canadian Institutes of Health Research and the Alberta Heritage Foundation for Medical Research for their ongoing support.

Competing interests: None declared.

References

1. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992;6:2311-22.

2. Rupprecht R, Hoelsboer F. Neuroactive steroids: mechanism of action and neuropsychopharmacological perspectives. *Trends Neurosci* 1999;22:410-5.

3. Baulieu EE, Robel P, Schumacher M. Neurosteroids: beginning of the story. *Int Rev Neurobiol* 2001;46:1-32.

4. Mellon SH, Deschepper CP. Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. *Brain Res* 1993;629:283-92.

5. Purdy RH, Paul SM. Potentiation of GABAergic neurotransmission by steroids. In: Baulieu EE, Robel P, Schumacher M, editors. *Contemporary endocrinology: neurosteroids. A new regulatory function in the nervous system*. Totowa (NJ): Humana Press; 1999. p. 143-53.

6. Mayo W, Vallee M, Darnaudry M, Le Moal M. Neurosteroids. Behavioral studies. In: Baulieu EE, Robel P, Schumacher M, editors. *Contemporary endocrinology: neurosteroids. A new regulatory function in the nervous system*. Totowa (NJ): Humana Press; 1999. p. 317-35.

7. Bitran D, Schiekh M, McLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. *J Neuroendocrinol* 1995;7:171-7.

8. Melchior CL, Ritzmann RF. Pregnenolone and pregnenolone sulfate, alone and with ethanol, in mice on the plus-maze. *Pharmacol Biochem Behav* 1994;48:893-7.

9. Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol* 1992;38:379-95.

10. Sajdyk TJ, Shekhar A. Excitatory amino acid receptor antagonists block the cardiovascular and anxiety responses elicited by gamma-aminobutyric acid A receptor blockade in the basolateral amygdala of rats. *J Pharmacol Exp Ther* 1997;283:969-77.

11. Reddy DS, Kulkarni SK. Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. *Brain Res* 1997;752:61-71.

12. Melchior CL, Ritzman RF. Dehydroepiandrosterone is an anxiolytic in mice on the plus maze. *Pharmacol Biochem Behav* 1994;47:437-41.

13. Romeo E, Strohle A, Spalletta G, di Michele F, Hermann B, Holsboer F, et al. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am J Psychiatry* 1998;155:910-3.

14. Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, et al. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proc Natl Acad Sci U S A* 1998;95:3239-44.

15. Uzunov DP, Cooper TB, Costa E, Guidotti A. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. *Proc Natl Acad Sci U S A* 1996;93:12599-604.

16. Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. *Proc Natl Acad Sci U S A* 1999;96:13512-7.

17. Semeniuk T, Jhangri GS, Le Mellédo JM. Neuroactive steroid plasma levels in patients with generalized anxiety disorder. *J Neuropsychiatry Clin Neurosci* 2001;13;396-8.

18. Heydari B, Le Mellédo JM. Decreased serum levels of preg-

nenolone sulfate in patients with generalized social phobia. *Psychol Med*. In press.

19. Ströhle A, Romeo E, di Michele F, Pasini A, Yassouridis A, Hoelsboer F, et al. GABAA receptor–modulating neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment. *Am J Psychiatry* 2002;159:145-7.

20. Wu FS, Gibbs TT, Farbs DH. Pregnenolone sulfate: a positive allosteric modulator at the NMDA receptor. *Mol Pharmacol* 1991;40:333-6.

21. Spivak B, Maayan R, Kotler M, Mester R, Gil-ad I, Shtauf B. Elevated circulatory levels of GABA antagonistic neurosteroids in patients with combat-related post-traumatic stress disorder. *Psychol Med* 2000;30:1227-31.

22. Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci U S A* 1991;88:4553-7.

23. Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and GABA-A receptor function after acute stress. *Neuroendocrinology* 1996;63:166-72.

24. Tait G, McManus K, Chrapko W, Lara N, Bellavance F, Le Mellédo JM. Neuroactive steroid response to pentagastrin in patients with panic disorder and healthy volunteers. *Psychoneuroendocrinology* 2002;27(4):417-29.

25. Givalois L, Grinevich V, Li S, Garcia-de-Yebenes E, Pelletier G. The octadecaneuropeptide-induced response of corticotropin-releasing hormone messenger RNA levels is mediated by $GABA_A$ receptors and modulated by endogenous steroids. *Neuroscience* 1998;85:557-67.

26. Patchev VK, Shoib M, Holsboer F, Almeida O. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience* 1994;62:265-71.

27. Patchev VK, Hassan AHS, Holsboer F, Almeida O. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology* 1996;15:533-40.

28. Patchev VK, Montkowski A, Rouskeva D, Koranyi L, Holsboer F. Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. *J Clin Invest* 1997;99:962-6.

29. Plotsky PM, Otto S, Sutton S. Neurotransmitter modulation of corticotropin-releasing factor secretion into the hypophysial portal circulation. *Life Sci* 1987;41:1311-7.

30. Charney DS, Breier A, Jatlow PI, Heninger GR. Behavioral, biochemical and blood pressure responses to alprazolam in healthy subjects: interactions with yohimbine. *Psychopharmacology* 1986;88:133-40.

31. Curtis GC, Abelson JL, Gold PW. Adrenocorticotropin-releasing hormone: changes in panic disorder and effects of alprazolam treatment. *Biol Psychiatry* 1997;41:76-85.

32. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress: elaborating and testing the concepts of allostasis and allostatic load. *Ann N Y Acad Sci* 1999;896:30-47.

33. Strohle A, Romeo E, Hermann B, Pasini A, Spalleta G, di Michele F, et al. Concentration of 3 alpha-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. *Biol Psychiatry* 1999;45:274-7.